TUMOR INITIATING CELLS: THE STEM CELL THEORY OF CANCER
Plan

- Stem cells
- The “stem cell” theory of cancer: tumor initiating cells
- The leukemia model
- Identifying cancer stem cells (CSC)?
- Limits of the model
STEM CELLS
Tissues are composed of a heterogeneous array of cells, with a hierarchical organization:
- during development
- in a given tissue

There are differences:
- in the degree of specialization
- the capacity of proliferation
- the capacity of self-renewal

Concept first development in hematology
Hierarchical organization of tissues

- Stem cell
  - Precursor/progenitor
  - (« transit amplifying cells »)
- Differentiated cells
Stem cells

- Essential features:
  - Undifferentiated cells
  - Self-renewal
  - Differentiation
  - Functional definition, a hidden property that must be demonstrated by functional tests
Classification of stem cells

- origin:
  - embryonic,
  - foetal
  - adult

- differentiation capacity
  - Unipotent SC
  - Multipotent SC
  - Pluripotent SC
Differentiation of stem cells

- Divisions:
  - asymmetric
  - stochastic model
The stem cell niche

- Microenvironment maintains the stem cell property
- A way to regulate stem cell numbers

Symetric division

Asymetric division

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CANCER STEM CELLS
Is a tumor homogeneous?

- Within a malignant tumor or among the circulating cancerous cells of a leukemia, there can be a variety of types of cells: **phenotypic heterogeneity** (beware: different from genetic heterogeneity)
- The stem cell theory of cancer proposes that among all cancerous cells, a few act as stem cells that reproduce themselves and sustain the cancer, much like normal stem cells normally renew and sustain our organs and tissues

http://ludwigcenter.stanford.edu/overview/theory.html

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Cancer Stem Cells (CSC)

- **Definition:**
  - theory: a cell with self renewal capacity, capable to produce a new tumor; theoretically, a single CSC could give rise to a new tumor.
  - Origin of CSC: only the SC live enough to accumulate the DNA modifications necessary for the malignancy to arise

- **Consequences:**
  - Functional test: a CSC will produce a tumor in an immunosuppressed mice; or can be genetically traced in mice cancer models
  - May display some markers of normal SC
  - Major potential clinical consequences
THE LEUKEMIA MODEL
Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell.

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The leukemia model

- Normal hematopoietic stem cells: CD34+ CD38-
- And in leukemia?
The leukemia model

- Graft into the immunosuppressed mice NOD/SCID
The leukemia model

- Graft into the immunosuppressed mice NOD/SCID
The leukemia model
The leukemia model
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Clonal evolution

1911
Rous discovers his sarcoma virus

1953
Furth and Kahn graft single mouse tumor cell

1966
Belanger and Leblond develop autoradiography and document stem cell hierarchies

1976
Pierce: teratomas contain pluripotent stem cells

1963
Till and McCulloch discover hematopoietic stem cells

1976
Multiple studies report stem-like cells in hematological malignancies

1975
Nobel Prize for Peyton Rous

1976
Knudson defines tumor suppressor 'two-hit' hypothesis

1976
Varmus and Bishop discover the Src protooncogene

1976
Nowell formulates the clonal evolution model of multihit carcinogenesis

1986
Weinberg and colleagues clone the first tumor suppressor Rb

1989
Nobel for Varmus and Bishop

1995
John Dick revives CSC theory by xenografting human AML

2003
Clarke demonstrates CSCs in breast cancer by xenografting

Cancer stem cells
IMPLICATIONS FOR TREATMENT

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Implication for cancer therapies

- If the concept of the CSC is true:
  - It would explain the heterogeneity of tumors
  - It would have wide implications for cancer therapies!
    - Only anti-cancer treatments killing CSC would prevent relapse
    - Industry: anti-CSC programs
Identification of Drugs Including a Dopamine Receptor Antagonist that Selectively Target Cancer Stem Cells

Eleftherios Sachlos,1 Ruth M. Risueño,1 Sarah Laronde,1 Zoya Shapovalova,1 Jong-Hee Lee,1 Jennifer Russell,1 Monika Malig,1 Jamie D. McNicol,1 Aline Fiebig-Comyn,1 Monica Graham,1 Marilyne Levadoux-Martin,1 Jung Bok Lee,1 Andrew O. Giacomelli,2 John A. Hassell,2 Daniela Fischer-Russell,1 Michael R. Trus,3 Ronan Foley,3 Brian Leber,3 Anargyros Xenocostas,4 Eric D. Brown,2 Tony J. Collins,1 and Mickie Bhatia1,2,*

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*Correspondence: mbhatia@mcmaster.ca
DOI 10.1016/j.cell.2012.03.049
Erosion of the chronic myeloid leukaemia stem cell pool by PPARγ agonists

Stéphane Prost¹, Francis Relouzat¹, Marc Spentchian², Yasmine Ouzegdouh¹, Joseph Saliba¹, Gérald Massonnet³, Jean-Paul Beressi⁴, Els Verhoeyen⁵,⁶, Victoria Raggueneau⁷, Benjamin Maneglier⁸, Sylvie Castaigne⁹, Christine Chomienne³, Stany Chrétien¹,¹⁰*, Philippe Rousselot³,⁹* & Philippe Leboulch¹,¹¹,¹²*
“Targeting self-renewal, an Achilles' heel of cancer stem cells”

Cancer treatment by differentiation

- Acute myeloblastic leukemia (AML3)
  - PML/RAR translocation: retinoic acid receptor
  - Treatment with all-trans retinoic acid (ATRA)
  - Poor prognostic ➔ Good prognostic

- AML and CD44
Ligation of the CD44 adhesion molecule reverses blockage of differentiation in human acute myeloid leukemia

RACHIDA-SIHEM CHARRAD¹, YUE LI¹, BERTRAND DELPECH², NICOLE BALITRAND³, DENIS CLAY¹, CLAUDE JASMIN¹, CHRISTINE CHOMIENNE³ & FLORENCE SMADJA-JOFFE¹


Article

Published online: 24 September 2006 | doi:10.1038/nm1483

Targeting of CD44 eradicates human acute myeloid leukemic stem cells

Liqing Jin¹,², Kristin J Hope¹,³, Qiongli Zhai², Florence Smadja-Joffe² & John E Dick¹

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IDENTIFYING CSC
IDENTIFYING AND ISOLATING CSC

1. Sorting based on cell surface markers
2. Colony/spheroid formation assay
3. Side-population assay
4. Assay based on ALDH activity
# CSC MARKERS

## Table 1 Surface markers used for the identification of CSCs

<table>
<thead>
<tr>
<th>Marker</th>
<th>Expression in healthy tissue</th>
<th>Marks cancer stem cells in</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19</td>
<td>Broadly on B lymphocytes</td>
<td>B cell malignancies</td>
</tr>
<tr>
<td>CD20</td>
<td>Broadly on B lymphocytes</td>
<td>Melanoma</td>
</tr>
<tr>
<td>CD24</td>
<td>Broadly on B cells; neuroblasts</td>
<td>Pancreas/lung cancer, negative on breast cancer</td>
</tr>
<tr>
<td>CD34</td>
<td>Hematopoietic and endothelial progenitors</td>
<td>Hematopoietic malignancies</td>
</tr>
<tr>
<td>CD38</td>
<td>Multiple stages of B and T cells</td>
<td>Negative on AML</td>
</tr>
<tr>
<td>CD44</td>
<td>Broadly on many tissues</td>
<td>Breast/liver/head and neck/ pancreas cancer</td>
</tr>
<tr>
<td>CD90</td>
<td>T cells, neurons</td>
<td>Liver cancer</td>
</tr>
<tr>
<td>CD133</td>
<td>Proliferative cells in multiple organs</td>
<td>Brain/colorectal/lung/liver cancer</td>
</tr>
<tr>
<td>EpCAM/ESA</td>
<td>Panepithelial marker</td>
<td>Colorectal cancer, pancreatic cancer</td>
</tr>
<tr>
<td>ABCB5</td>
<td>Keratinocyte progenitors</td>
<td>Melanoma</td>
</tr>
</tbody>
</table>

ABCB5, ATP-binding cassette transporter B5; EpCAM, epithelial cell adhesion molecule; ESA, epithelial-specific antigen. Table is adapted from ref. 29.
Side population : SP

- Hoechst 33342:
  - cell-permeable DNA stain excited by ultraviolet light and emits blue fluorescence at 460-490 nm
  - Binds to DNA
  - Actively transported out of the cell via ABC transporters

- Negative population “SP” is enriched in SC
Aldehyde dehydrogenases: group of enzymes for detoxification of exogenously and endogenously generated aldehydes.

- ALDH+ cells are enriched in SC

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Spheroids

- Floating cell aggregates
- Phenotypic heterogeneity
- Long term growth (with passages): only if CSC

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FUNCTIONAL TESTS

NOD/SCID

Three injections of 4,000 cells

90 days
First palpable

170 days
Analysis

1/3 tumours

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NATURE|Vol 456|4 December 2008
ARE CSC RARE?
Efficient tumour formation by single human melanoma cells

Elsa Quintana¹*, Mark Shackleton¹*, Michael S. Sabel², Douglas R. Fullen³, Timothy M. Johnson⁴ & Sean J. Morrison¹

cancers. Studies on diverse cancers, including melanoma, have indicated that only rare human cancer cells (0.1–0.0001%) form tumours when transplanted into non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice. However,
<table>
<thead>
<tr>
<th>Patient</th>
<th>Mouse strain</th>
<th>Co-injection</th>
<th>Number of tumours / number of injections (cells per injection)</th>
<th>Melanoma-initiating cell frequency (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NOD/SCid</td>
<td>Vehicle</td>
<td>0/3, 0/6, 0/3</td>
<td>(&lt;1/60,000)</td>
</tr>
<tr>
<td>481</td>
<td>NOD/SCid</td>
<td>Matrigel</td>
<td>6/6, 4/6, 1/5*</td>
<td>(1/2–1/13)</td>
</tr>
<tr>
<td>491</td>
<td>NOD/SCid</td>
<td>Vehicle</td>
<td>0/3, 0/6</td>
<td>(&lt;1/5,100)</td>
</tr>
<tr>
<td></td>
<td>NOD/SCid</td>
<td>Matrigel</td>
<td>6/6, 1/6, 1/15*</td>
<td>(1/6–1/40)</td>
</tr>
<tr>
<td>492</td>
<td>NOD/SCid</td>
<td>Vehicle</td>
<td>3/3, 3/6, 0/6</td>
<td>1/7,300</td>
</tr>
<tr>
<td></td>
<td>NOD/SCid</td>
<td>Matrigel</td>
<td>6/6, 2/6, 1/11*</td>
<td>(1/2,400–1/22,300)</td>
</tr>
</tbody>
</table>

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ARE CSC RARE?

- CSC melanoma:
  - 1/1 000 000 (Nature. 2008 Jan 17;451(7176):345-9)
  - 1/4 (Nature. 2008 Dec 4;456(7222):593-8)

- Depends on the functional test!!!

- Concept of tumor initiating cells (TICs)
TARGETING CSC BUT NOT SC

- If CSC have a similar metabolism to SC, treating CSC may target normal SC
OTHER MODELS
OTHER MODELS?

Combination of CSC and stochastic model

Mutation

Minor clones

Major clone

:: CSCs

:: Non-CSCs

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Sugihara et al. Int J Can 2013
CLONAL EVOLUTION OF AML

Differentiation reversibility

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Sugihara et al. Int J Can 2013
EMT?

CSCs

EMT etc.

Non-CSCs

Sugihara et al. Int J Can 2013
OTHER (NOT EXCLUSIVE)

- Differentiation blockage
- Reproduction of fetal or embryonic properties
- New phenotype 🌼
CTC!
Circulating tumor cells

- Circulating tumor cells = CTC
CONCLUSION

- High stakes
- Difficult to formally prove the CSC theory
- New pathways of research:
  - Genetic tracing (mouse models)
  - Single cell mRNAseq and DNAseq
Three recent reviews:

